Jensen, Kathleen

From: Jensen, Kathleen
Sent: Friday, April 19, 2013 4:18 PM
To: 'Constantine, Lisa A'
Cc: Ankley, Gerald; Boucher, Joseph F.
Subject: RE: 17α-trenbolone data

Hi Lisa,

The use of a paired spawning design allowed us to relate potential changes in biochemical endpoints, collected on a per fish basis, to reproduction data obtained from individual females.

Kathy

From: Constantine, Lisa A [mailto:Lisa.A.Constantine@pfizer.com]
Sent: Friday, April 19, 2013 12:24 PM
To: Jensen, Kathleen
Cc: Ankley, Gerald; Boucher, Joseph F.
Subject: RE: 17α-trenbolone data

Hi Kathy,

Thanks for getting back to me and I hope you are feeling better.

I appreciate your offer to provide the fecundity data from the first study. Although I don't believe it will be necessary at this time, should this change, I will be in touch. In the meantime, I have 1 additional question.

Would you mind clarifying why 8 pairs were used in the second study instead of 4 females and 2 males per spawning group used in the first study and as recommended in the Ankley protocol and in OECD 229 guideline?

Thanks

Kind regards,

Lisa

From: Jensen, Kathleen [mailto:Jensen.Kathleen@epa.gov]
Sent: Thursday, April 18, 2013 8:02 AM
To: Constantine, Lisa A
Cc: Ankley, Gerald; Boucher, Joseph F.
Subject: RE: 17α-trenbolone data

Hi Lisa,

I'm sorry for the delay in responding...between being out sick for a few days and late spring snowstorms closing the lab, I am a bit behind. At any rate, please see answers to your questions below:

1. The Toxicity Relationship Analysis Program (TRAP, Version 1.00) was used to determine the reported EC50 value. More information for TRAP can be found at http://www.epa.gov/medatwrk/Prods_Pubs/trap.htm
2. The rationale for limiting the fecundity data set to the last 2 weeks for determination of the EC50 value was based on results obtained in the first α –trenbolone study that we conducted (referred to as Study 1

in the paper). In this study, there was no egg production observed in any trenbolone treatments (0.13-7.1 µg/L) after the first few days of the study. There were a few spawns observed within the first week of exposure, primarily a reflection of where an individual female happened to be relative to the previous spawn. After this initial period, however, no spawning was observed for the duration of the exposure. Consequently, our EC50 determination of the fecundity data in the second study was based on data collected after the first week of exposure as we felt that duration best reflected the effect of trenbolone on fathead minnow spawning. The fecundity data from the first study was not reported in the paper but I would be happy to share it if you are interested.

3. There was a need to address three mortalities (2 male and 1 female) in the fecundity data set. We did not use spawning data collected from these spawning pairs due to the death of one fish in the pair.

Again, please let me know if you would like to see the fecundity data from the first study or have any additional questions.

Kathy

From: Constantine, Lisa A [Lisa.A.Constantine@pfizer.com]
Sent: Thursday, April 11, 2013 12:04 PM
To: Jensen, Kathleen
Cc: Ankley, Gerald; Boucher, Joseph F.
Subject: RE: 17α-trenbolone data

Hi Kathy,

Thanks again for provided these data. Upon review, we do have a few questions:

1. Would you please provide the method used to determine the EC50 value.
2. Clarify the rationale for limiting the fecundity data set to the last 2 weeks vs the full 21 days when determining the EC50 value.
3. Within the fecundity data set, was there a need to address the male mortalities and if so, how was this handled.

I appreciate your time and assistance and look forward to receiving your responses.

Kind regards,

Lisa

From: Jensen, Kathleen [mailto:Jensen.Kathleen@epa.gov]
Sent: Monday, March 25, 2013 4:10 PM
To: Constantine, Lisa A
Cc: Ankley, Gerald
Subject: 17α-trenbolone data

Hi Lisa,

Gary asked me to provide you with the data from our 17α-trenbolone study. The data that I have attached is from the second study discussed in the paper. An initial study was conducted at higher trenbolone concentrations and, based on effects observed in that study (i.e., no spawning in any treated tanks and 100% masculinization of females), a second study was conducted at lower trenbolone concentrations. However, we did report analytical data from both studies in the paper (Table 1). I have attached two files:

1. Fecundity Data (daily egg counts) - As summarized in the paper, there were four trenbolone treatments plus a control. There were four replicate tanks per treatment, each containing 2 pairs of adult fathead minnows separated by a mesh

screen. In the attached Excel file, you will find the treatment/replicate/pair data is organized in columns along with exposure duration (21 days). The numbers in the cells represent the total number of eggs spawned each day. Please note there were several mortalities and one replicate pair where an immature male was incorrectly identified as female.

2. Sample Data - data collected at termination of the study on a per fish basis: body weight, gonad weight, plasma vitellogenin and steroids (E, T, 11-KT), GSI, and tubercle score.

Please let me know if you have any questions or would like to see additional data.

Kathy

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